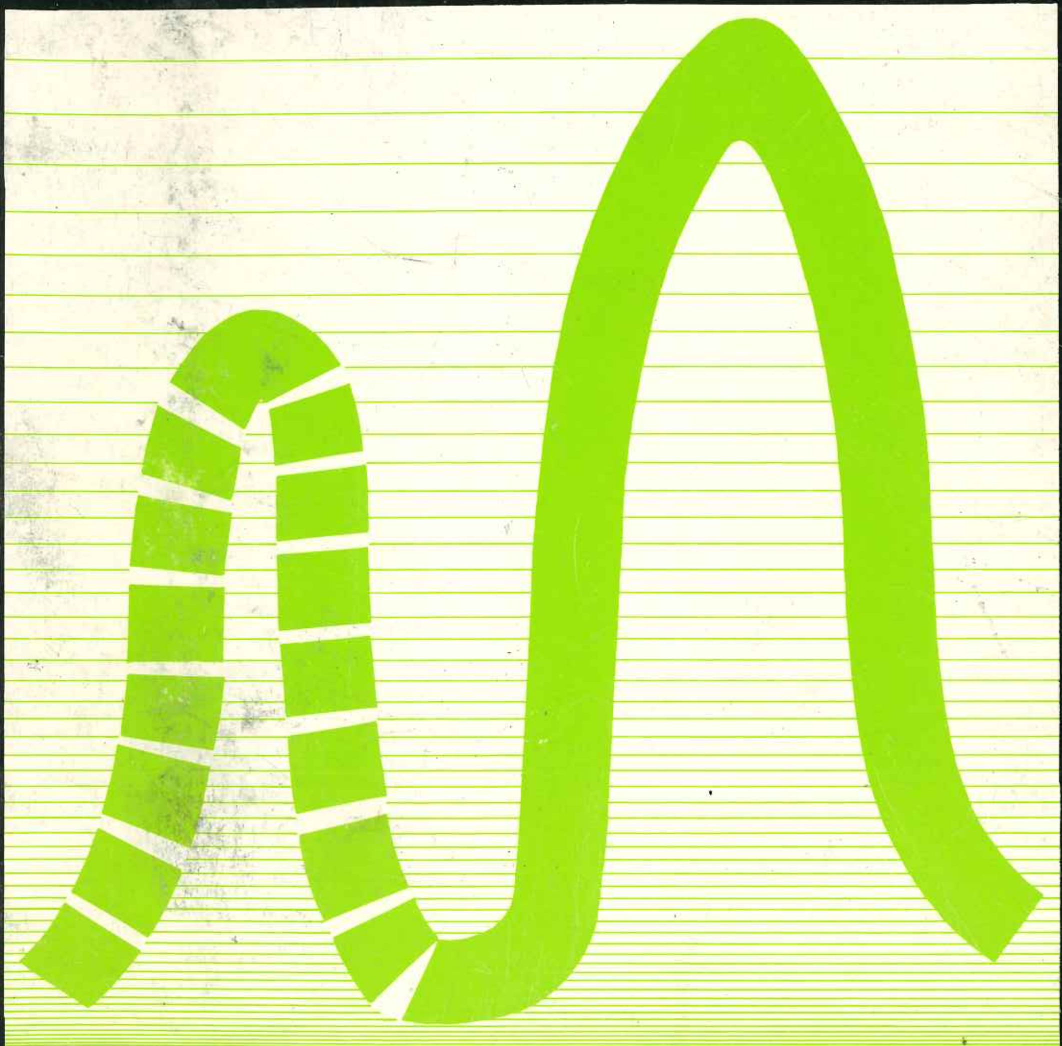
Sephacryl S-200 Superfine

For high performance gel filtration



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Sephacryl S-200 Superfine

Pharmacia Fine Chemicals, the makers of Sephadex®, are pleased to introduce Sephacryl® S-200 Superfine, a unique, high resolution chromatographic medium for gel filtration of proteins, nucleic acids, polysaccharides and other biopolymers.

Sephacryl S-200 Superfine is a new kind of gel filtration medium which combines a highly porous gel structure with excellent chemical and physical stability. Sephacryl is preswollen and ready to use in both analytical and preparative applications. Because of its stability and small bead-size you can

- use flow rates of up to 30 cm/hour to separate proteins in the molecular weight range 5,000—250,000 daltons
- obtain high resolution with reduced zone spreading
- use high flow rates for rapid column packing and equilibration
- sterilize Sephacryl repeatedly by autoclaving.

Chemical and physical properties

Sephacryl S-200 Superfine is prepared by covalently cross-linking allyl dextran with *N, N'*-methylene bisacrylamide to give a rigid gel with a carefully controlled range of pore sizes*. A hypothetical partial structure is shown in Figure 1. A small number of carboxyl groups may be present.

The wet bead diameter is 40—105 μm, average bead diameter approximately 70 μm.

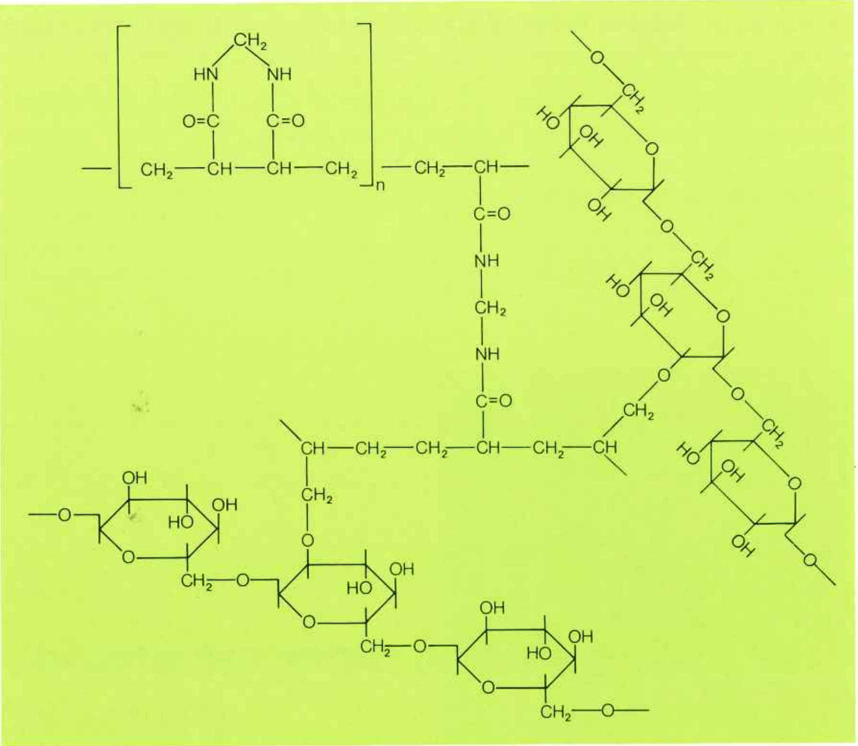

Fig. 1. Hypothetical partial structure of Sephacryl.

* Patent pending

Chemical stability

Sephacryl is insoluble in all solvents unless it is chemically degraded. It may be used in the range pH 3—11. At lower pH, limited hydrolysis of the dextran chains may occur. Sephacryl has been treated with 0.2 M NaOH for 100 hours at room temperature without significant effect on flow rates or porosity. Sephacryl may be used with eluents containing detergents, e. g. SDS, and with structure-breaking media such as 6 M guanidine hydrochloride.

Physical stability

Its unique chemical structure makes Sephacryl S-200 Superfine exceptionally rigid. Excellent flow rates are obtained in both large and small columns (fig. 2). Flow rate versus pressure curves obtained with Sephacryl S-200 Superfine in Pharmacia Columns K 16 and K 26 at bed heights up to approximately 100 cm are generally similar to the curve shown for a 30 cm bed in Column K 16/40.

Maximum flow rates of 50 cm/hour have been obtained in a "Stack" segment, KS 370, bed height 15 cm, and 13.5 cm/hour has been obtained in a Column K 100/100, bed height 87 cm.

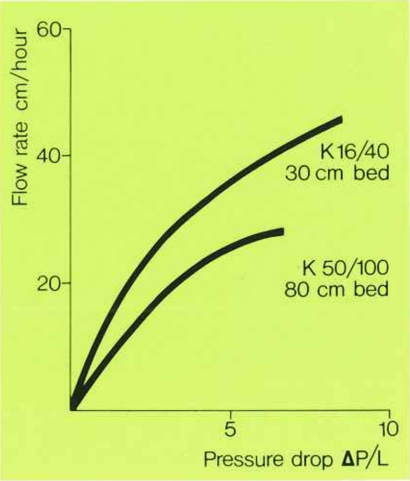

Fig. 2. Flow rates obtained with Sephacryl S-200 Superfine in 0.9 % NaCl solution. Flow rates were measured in the Pharmacia Columns indicated.

Thermal stability

Sephacryl does not melt and may be autoclaved repeatedly at pH 7, 120° C without significantly affecting its chromatographic properties.

Stability in organic solvents

Sephacryl is stable in a variety of organic solvents and replacement of the water in the gel with an organic solvent has a much smaller effect on pore size than it has with Sephadex. A method for transferring Sephacryl from water to organic solvents is described in the experimental section (see page 13). Gel volumes obtained with various organic solvents, starting from 100 ml of Sephacryl sedimented in water, are shown in Table 1.

Table 1

Organic solvent	Approximate vol. from 100 ml sedimented gel in water
Dimethyl sulphoxide	100
Dimethyl formamide	100
Ethyl acetate	70
Chloroform	70
n-Heptane	65
Tetrahydrofuran	65
Acetone	65
Toluene	60

Chromatographic properties

Selectivity

Sephacryl S-200 Superfine has an exclusion limit of approximately 250,000 daltons for globular proteins and approximately 80,000 daltons for dextrans. Selectivity curves are shown in Figure 3.

Adsorption

Sephacryl adsorbs Blue Dextran 2000 at pH below 7 at low ionic strength. At higher pH adsorption effects are small. Aromatic adsorption effects have been demonstrated for nucleosides and aromatic amino acids.

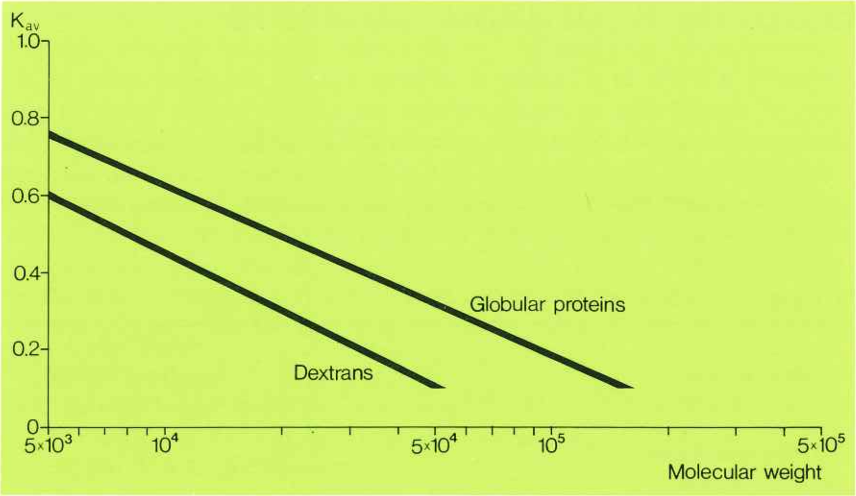

Fig. 3. Selectivity curves for Sephacryl S-200 Superfine.

Applications

Sephacryl S-200 Superfine gives excellent resolution of proteins, polysaccharides and other biopolymers. Because Sephacryl is mechanically strong, it can be used even in large preparative columns and still give good flow rates.

High speed gel filtration

In many cases Sephacryl S-200 Superfine will be found to generate more resolution than is actually needed to achieve the desired separation. This excess resolution can be traded-off for increased flow rates and decreased separation times. The chromatograms shown in Figure 4 illustrate the effects of flow rate on the resolution of a three-component model system.

Sephacryl S-200 Superfine was packed in a Pharmacia Column K 26/100 (bed height 85 cm) and equilibrated at a flow rate of 3.5 ml/min with Tris/HCl buffer solution (pH 8.0, 0.1 M) containing NaCl (0.5 M). The sample contained IgG (8 mg), transferrin (6 mg) and α-chymotrypsinogen (6 mg) in 0.8 ml buffer solution. Flow rates are given in Figure 4.

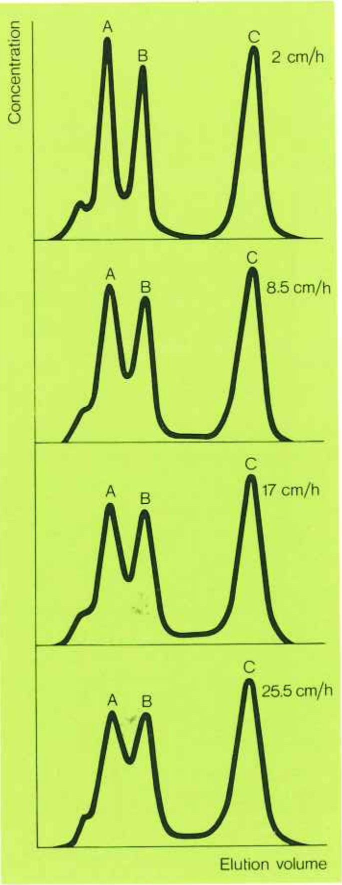

Fig. 4. Gel filtration of a mixture of IgG (A), transferrin (B) and α-chymotrypsinogen (C) on Sephacryl S-200 Superfine. Pharmacia Column K 26/100; bed height, 85 cm; eluent, Tris/HCl buffer solution (pH 8.0, 0.1 M) containing NaCl (0.5 M); flow rates as shown. (Work from Pharmacia Fine Chemicals.)

The slowest flow rate gives the highest resolution as is usual in gel filtration. This is clear from a comparison of the separation between the first two main peaks, IgG and transferrin. However, α-chymotrypsinogen is still completely resolved from the high molecular weight components when the flow rate is

25.5 cm/hour (135 ml/hour). The separation of transferrin from α-chymotrypsinogen could have been further speeded up by reducing the bed height from 85 cm to about 20 cm, thereby giving a separation time of 35 minutes.

Details of how to optimize the conditions for a given separation are given in the section "Experimental design in gel filtration" (pages 10—12).

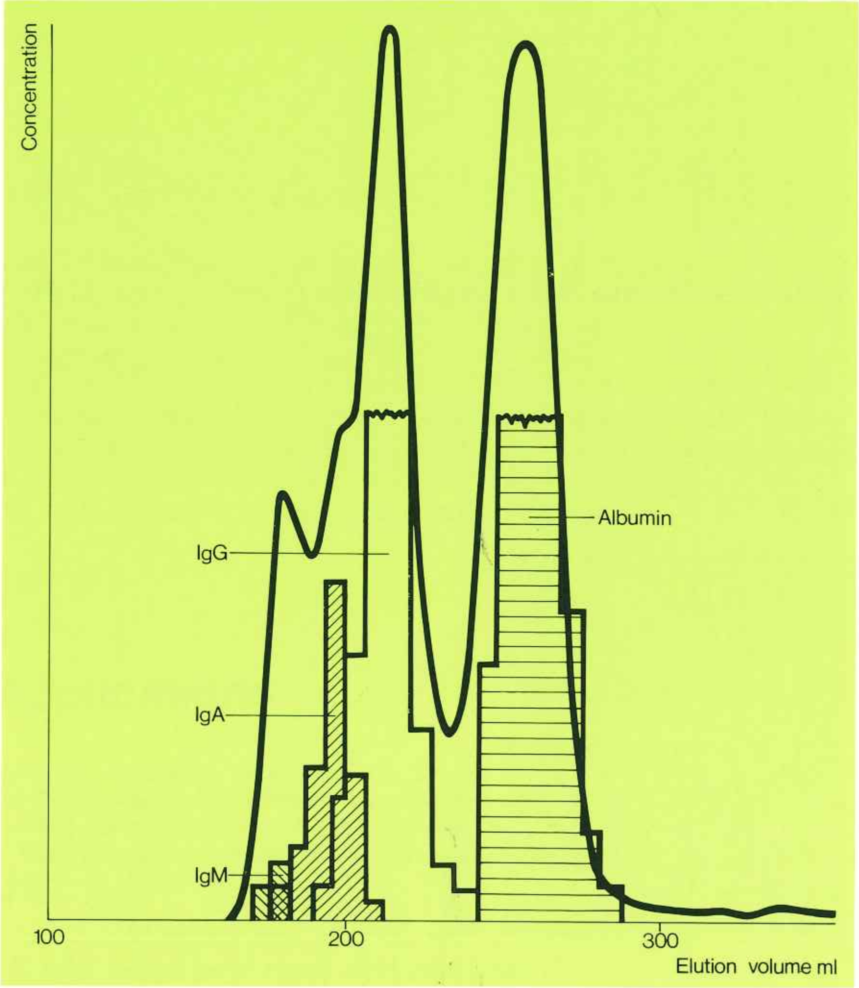

Fig. 5. Gel filtration of serum proteins on Sephacryl S-200 Superfine. Pharmacia Column K 26/100; bed height, 85 cm; eluent, Tris/HCl buffer solution (pH 8.0, 0.1 M) containing NaCl (0.5 M); flow rate, 11.2 ml/hour. (Work from Pharmacia Fine Chemicals.)

Human serum proteins

Typical results for the fractionation of human serum proteins using Sephacryl S-200 Superfine are shown in Figure 5. A Column K 26/100 was packed (bed height 85 cm) and equilibrated with Tris/HCl buffer as described above. The sample was 2 ml human serum and the column was eluted at a flow rate of 11.2 ml/hour (2.1 cm/hour). Fractions were collected and examined for IgM, IgA, IgG and serum albumin by quantitative immunodiffusion. Elution positions are shown in Figure 5. IgG is completely separated from both IgM and serum albumin.

Cytosol from *Cytophaga jonsonii*

Washed cells of *Cytophaga jonsonii* (10 g wet weight) were disrupted in a French press. Cell debris was removed by centrifugation and the supernatant cytosol was treated with ribonuclease and deoxyribonuclease to reduce the viscosity. Cytosol (6 ml) was applied to a column of Sephacryl (K 26/100, bed height 94 cm) packed and equilibrated with Tris/HCl buffer as described above. The column was eluted at a flow rate of 56 ml/hour (10.6 cm/hour). The effluent was monitored at 254 nm and fractions were assayed for α-glucosidase activity (fig. 6). Spectral analysis of material eluting just after the total volume showed it to be degradation products of nucleic acids.

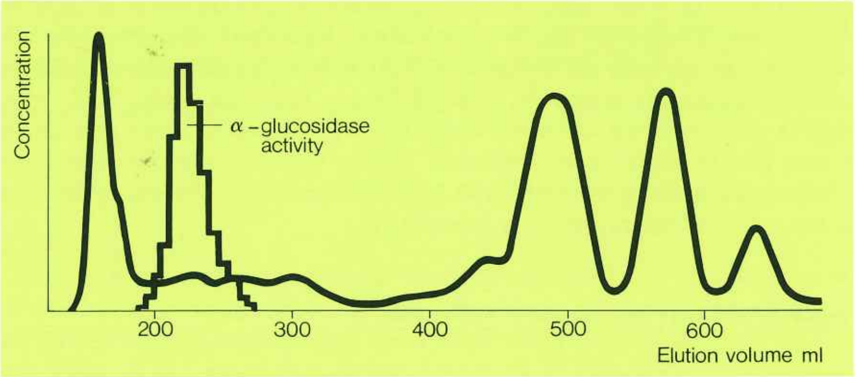

Fig. 6. Gel filtration of cytosol on Sephacryl S-200 Superfine. Pharmacia Column K 26/100; bed height, 94 cm; eluent, Tris/HCl buffer solution (pH 8.0, 0.1 M) containing NaCl (0.5 M); flow rate, 56 ml/hour. (Work from Pharmacia Fine Chemicals.)

Affinity chromatography

Sephacryl S-200 Superfine can be activated with CNBr (1) and used to couple ligands containing amino groups. The coupling efficiency for small ligands can be expected to be similar to that obtained with Sepharose® 4B, but coupling of proteins to Sephacryl is only about 10 % or less of that obtained with Sepharose 4B.

Experimental design in gel filtration

The main objective of any chromatography experiment is to achieve a separation. For some applications, e.g. desalting operations, it is easy to obtain both the required degree of separation and a short separation time (2). At the other extreme, the complete resolution of biological fluids as complex as blood serum can seldom be attained by any single technique. Such separations are necessarily time-consuming. There are, however, many separations which can be significantly improved by the correct choice of chromatographic conditions. Sephacryl S-200 Superfine opens up new possibilities for achieving good separations in a shorter time.

Resolution and speed

For a given gel filtration medium, the resolution obtained will depend on

1. a well-packed chromatographic bed in a column of correct design
2. bed length (L)
3. flow rate.

Resolution, R_S, is defined by

$$R_S = \frac{\text{distance between zone centres}}{\text{zone width}}$$

Resolution is proportional to $\sqrt{L}$ and, under conditions which are usually encountered in gel filtration, decreases with increase in flow rate. The maximum resolution is obtained with a long column and a low flow rate and the fastest run is obtained with a short column and a fast flow rate. Good resolution and short running times may thus appear to be basically incompatible.

However, if peaks are well separated at a low flow rate using a long column, the excess resolution may be traded off for speed. The flow rate can be increased and a shorter column can be used.

Optimization

The behaviour of a three-component model system of IgG, transferrin and α-chymotrypsinogen can be used to show how a separation can be speeded up (fig. 7). IgG and transferrin are separated at the lowest flow rate. However, this separation can probably not be speeded up significantly without serious loss of resolution. Transferrin is well separated from α-chymotrypsinogen and this particular separation can be speeded up by a factor of 50.

In the first stage of the optimization, increasing the flow rate, the time for separating α-chymotrypsinogen from transferrin is reduced from about 30 hours to about 2½ hours. The resolution (R_S) is still 2.25.

The bed height (L_1) required to give $R_S = 1$ can be calculated from

$$L_1 = \frac{L}{R_S^2}$$

where R_S is the resolution obtained with a column length L. In this example $L_1 = 17$ cm. Reducing the bed height to about 20 cm should still give adequate resolution of α-chymotrypsinogen and transferrin. The result obtained with a bed height of 19.5 cm at 24.5 cm/hour is shown in Figure 7. The separation time for α-chymotrypsinogen and transferrin has been reduced from 30 hours to 35 minutes. For a full discussion of the optimization of gel filtration see "The dynamics of chromatography", J. C. Giddings (Marcel Dekker, N.Y. 1965) Part 1, Principles and theory.

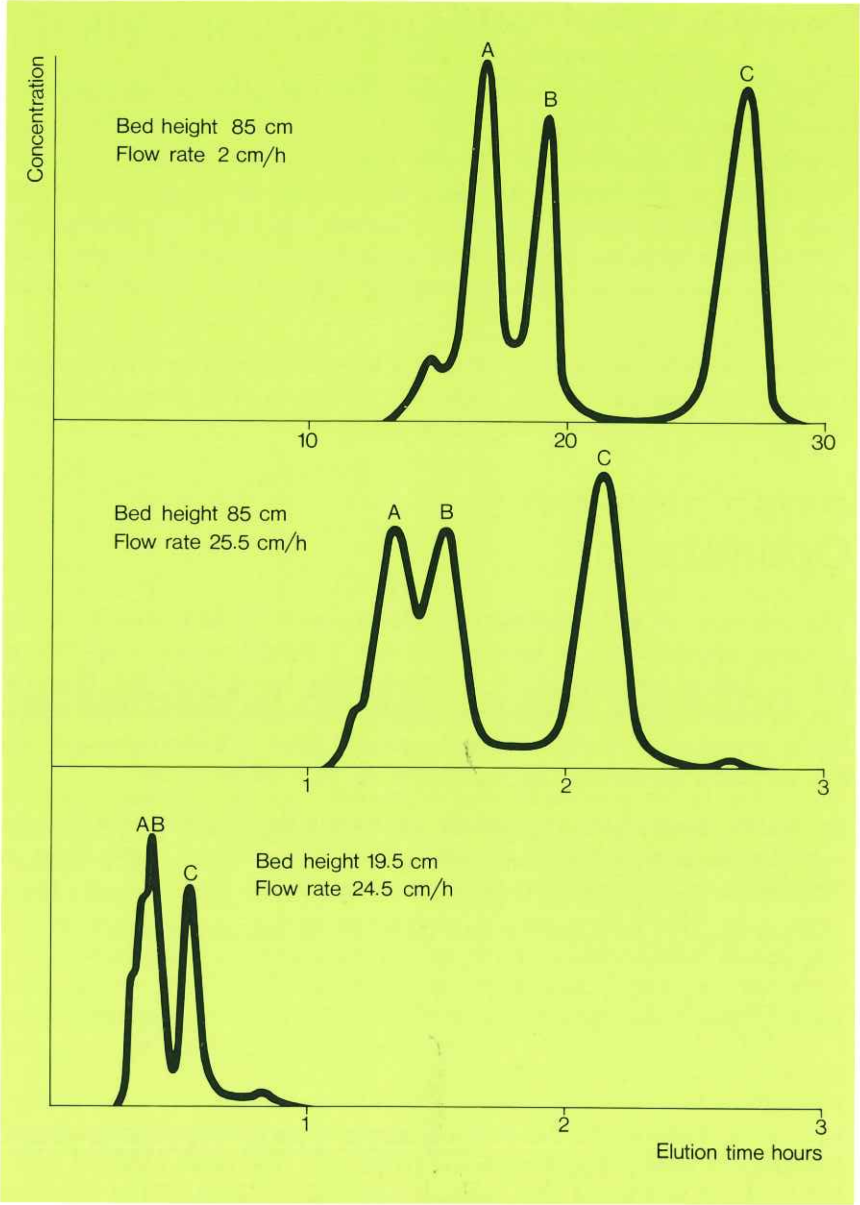

Fig. 7. Gel filtration of a mixture of IgG (A), transferrin (B) and α-chymotrypsinogen (C) on Sephacryl S-200 Superfine. Pharmacia Column K 26; eluent, Tris/HCl buffer solution (pH 8.0, 0.1 M) containing NaCl (0.5 M); bed heights and flow rates as shown. (Work from Pharmacia Fine Chemicals.)

Experimental methods

Preparation of the gel for use in aqueous media

Sephacryl S-200 Superfine is supplied preswollen as a suspension in distilled water containing 0.02 % sodium azide as a bacteriostatic agent. The suspension is too thick to be poured into a chromatographic column directly and it should first be diluted with eluent to a consistency which allows the easy escape of air bubbles. Final equilibration with the eluent is most efficiently carried out in the column during bed stabilization.

Preparation of the gel for use in organic solvents

The aqueous medium in which Sephacryl is supplied can be exchanged for a variety of organic solvents. To ensure efficient replacement of the water by the required solvent, the transfer must be made through a graded series of solvent mixtures. Thus to transfer Sephacryl from one pure solvent (A) to another pure solvent (B), the gel is transferred first to 70 % A/30 % B then to 30 % A/70 % B and finally to pure B. If A and B are not mutually miscible, the transfer is made via an intermediate solvent e.g. Sephacryl is transferred from water to chloroform via acetone (fig. 8).

Transfer the required amount of Sephacryl to a sintered glass Buchner funnel and remove the excess aqueous medium by gentle suction. Add the next solvent (see fig. 8) and resuspend the gel by gentle stirring. Suck off the excess solvent and resuspend in the same solvent. Repeat the process with the next solvent of the series, allowing two resuspensions and proceed until the required solvent composition is reached.

Note that the gel volume may be reduced by up to 45 % on transfer to organic solvents (Table 1).

Gel filtration in aqueous media

Sephacryl S-200 Superfine may be packed in the same way as Sephadex. The rigid structure of Sephacryl enables packing and equilibration to be carried out at high flow rates. For the best results packing should be carried

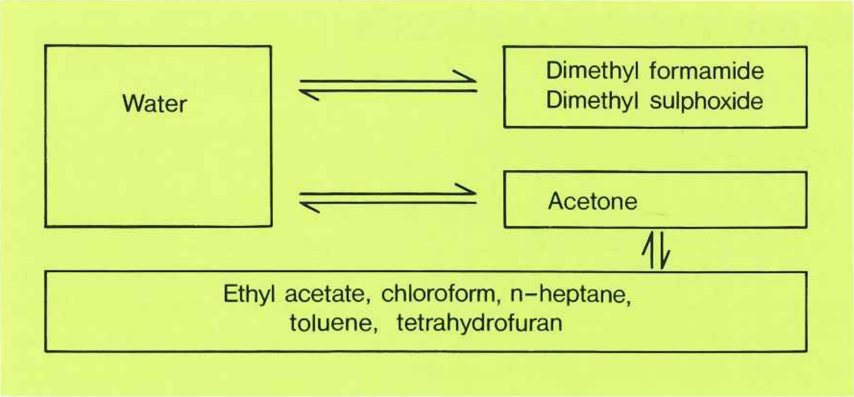
Water
Dimethyl formamide
Dimethyl sulphoxide
Acetone
Ethyl acetate, chloroform, n-heptane, toluene, tetrahydrofuran

Fig. 8.

out at a flow rate of approximately 40 cm/hour, which is equivalent to 3.5 ml/min in Column K 26 or 1.3 ml/min in Column K 16, using the Pharmacia Peristaltic Pump P-3. Columns K 50 should be packed at a flow rate of approximately 10 ml/min.

Details of experimental methods for packing, sample preparation and sample application are given in the handbook "Sephadex — Gel filtration in theory and practice" which is free on request. Pharmacia columns and other chromatographic equipment have been specially designed for chromatography of labile biological substances and are recommended for reproducible results.

Cleaning

Sephacryl S-200 Superfine may be readily cleaned by treatment with 0.2 M NaOH or non-ionic detergents.

Storage

Sephacryl S-200 Superfine should be stored as a suspension. Microbial growth may occur in the suspension medium, particularly in the presence of phosphate ions, but Sephacryl itself is very resistant to microbial degradation. Sodium azide (0.02 %), chloroform, trichlorobutanol or butanol (0.5 %) may be added to the suspension to prevent contamination.

Availability

Sephacryl S-200 Superfine is available in packs of 750 ml as a suspension in distilled water containing 0.02 % sodium azide.

References

1. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature 214 (1967) 1302—1304,* Axén, R., Porath, J., Ernback, S.
2. A rapid procedure for desalting and buffer exchange. *Separation News, September (1972),* Pharmacia Fine Chemicals AB.

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June 1976-1 Printed in Sweden by Rahms i Lund

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